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RECENT STUDIES UPON IMMUNITY.

BY

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I.

THE evolution of our bacterial knowledge has been so rapid during the last few years, that already the ideas prevalent early in the eighties are matters of ancient history, and the theories with regard to bacterial action and the nature of immunity which then obtained are wholly out of date. Yet these theories were useful in their day, and, if possessing no other value, they are useful now as marking the progressive advances of a young science.

Based upon very scanty observations, these older theories were disproved so soon as they were put to the experimental test. The "exhaustion" theory of Pasteur and Klebs, that bacteria use up certain materials in the economy, and that consequently further invasion and proliferation is prevented by the absence of the food material necessary for the growth of these bacteria, was shown to be insufficient when it was determined that the bacilli of anthrax would grow easily in the body fluids of animals rendered immune to the disease, and not only in the removed fluids, but also, under certain conditions, within the bodies of the same animals. And Chauveau's "retention" theory, which owed its origin to the fact that microbes grown outside the organism tend to be destroyed by the products of their metabolism, and which supposed that products of bacterial growth present in the fluids of the body prevent a second development of the bacteria, was found to be equally weak. It is in the highest degree improbable that such soluble substances should not be gradually excreted during the course of a very few days or weeks. But apart from this antecedent improbability, a further difficulty arises from the fact already mentioned, that microbes can grow easily in the removed body fluids of animals rendered refractory. Even if it be taken that the bacterial products are insoluble, then, like mercury, arsenic, and other substances which remain unexcreted, they must be stored up in certain organs, and the immunity conferred by them can be but local, confined to the organ or organs in which they are deposited; it cannot be generalised.

* In reprinting this article I have taken the opportunity to make one or two verbal alterations where it seemed to me that the original might be considered ambiguous.

And here must be mentioned another of the earlier theories, which may be said to date back to Eichhorn in 1829, and which has received more recent support from Buchner (1877) and Wolffberg (1885). This may be termed the theory of local immunity, and supposes that during infection the regions attacked by any specific disorder are so affected that the weaker cells are destroyed, while those which have survived the bacterial invasion transmit their resistant properties to their descendants. But granting that this occurs locally, in diseases which have a purely local manifestation, the supposition does not explain the immunity acquired by unaffected parts of the same organ. Why, for example, in ordinary vaccination, is not only the vaccinated area but the whole skin and mucous membrane rendered resistant to variola? And assuredly the theory cannot be applied to explain what may occur in connection with diseases like anthrax, in which subcutaneous inoculation of the attenuated virus, the bacilli remaining localised, suffices not only to give immunity against the cutaneous malady, but also prevents the onset of the disease either by way of the respiratory or of the intestinal tract.

These earlier theories must, therefore, be dismissed; some wider generalisation is demanded, a generalisation resting upon a more solid basis of facts, and, as the discussion at the recent Hygienic Congress in London abundantly proved, the question at present exciting the greatest interest among bacteriologists is whether Metchnikoff's cellular theory can adequately explain the known phenomena of the cure of infectious disease and the production of immunity,* or whether, in addition to, or apart wholly from the action of cells, the action of the bodily humours must be invoked. Although the enthusiastic reception accorded to Metchnikoff at the Burlington House meeting may have been but a tribute to his ceaseless energy, a spontaneous and ungrudging acknowledgment of the respect due to him for his laborious studies upon the subject during the greater part of the last ten years, yet the heartiness of the welcome seemed almost to indicate that the bacteriological world is now nearly prepared to accept his theory with few reservations, and that, in fact, among those competent to weigh the evidence, phagocytosis has almost won the day.

It is unnecessary here to detail the observations upon intracellular digestion in the invertebrata and lower vertebrata which led Metchnikoff to study phagocytosis in its relation to the prevention of bacterial invasion;

* The process of cure and resolution of infectious disease and the processes concerned in the production of immunity are of the same order of affairs; it is impossible to draw any sharp line of demarcation between the two. The conferment of immunity by means of attenuated virus is attended by local inflammatory action, and often by a febrile state of short duration—such as Gamalela has shown to be the inevitable attendant of successful inoculation of anthrax in sheep, and as, to come nearer home, constantly attends successful vaccination against variola; and similar disturbances attend the inoculation of powerful virus in animals already made refractory. Thus, to quote Metchnikoff, "*immunity is most often but recovery in operation from the very onset of a disease.*"

nor need I give a *resumé* of the separate researches of later years. It will be sufficient to state his theory as it at present stands, amplifying such portions of it as are seen to need fuller treatment. And the theory may be stated as follows:—

(1) That two forms of leucocytes, present in the blood and lymph, the large uninuclear "macrophages" and the smaller "microphages" which tend to the multinuclear, are, under certain conditions, capable of incepting such bacteria as have gained entry into the system.

(2) That the splenic corpuscles, endothelial cells, and sundry other cells of mesodermal origin, possess the same property, though it is called into action to a less extent.

(3) That these phagocytes incept and destroy living and active microbes.

(4) That the more acute the disease, the less the phagocytosis that can be determined; the more chronic, the more extensive the phagocytosis; or otherwise: the more virulent and rapidly proliferating the microbes, the less the potentiality of the phagocytes to incept and destroy them, and *vice versa*.

(5) That this potentiality of the phagocytes depends upon the "chemiotaxis" exerted by the products of bacterial growth. In the case of the more virulent microbes the leucocytes are repelled from the focus of infection; there is a *negative* chemiotaxis, and thus, in the absence of phagocytosis, the proliferation of the microbes takes place without hindrance; whereas, the less virulent microbes and their products attract the leucocytes; they exert a *positive* chemiotaxis, so that there is an emigration of leucocytes through the capillary walls to the focus of infection, and the leucocytes taking up the microbes arrest the infective process.

(6) That the leucocytes and other mesodermal cells may become accustomed, and eventually attracted, to substances by which at first they are repelled, and thus a negative may be transformed into a positive chemiotaxis. Hence, as in acute zymotic diseases, the microbes may at first rapidly proliferate, and phagocytosis manifest itself only after a certain period, leading then to resolution of the disease.

(7) That the cells having once acquired positive chemiotactic properties in relation to the products of any specific microbe retain and transmit these properties through a series of cell generations, the length of which varies according to the microbe, the extent of the primary reaction, and the idiosyncrasies of the individual.

(8) That, consequently, immunity and the cure of disease are mainly brought about by the activity of special cells, the phagocytes,—being primarily dependent upon the attraction existing between these cells and the products of bacterial metabolism.

(9) Yet while this is the main factor it is quite possible, nay, probable,

that others, with the extent and nature of whose action we are at present unacquainted, aid in combating bacterial invasion. Among these may be mentioned the febrile reaction.

The wise discernment that further observations and the consideration of a greater number of facts may lead to an expansion or modification of the theory has, it would seem, prevented Metchnikoff from placing it in a succinct finite form, and so fossilising it. However, I believe that the above, while not Metchnikoff's "ipsissima verba," is a full and correct statement of his theory as it stands at present. In his last "Etude sur l'immunité," which has come into my hands after going to press, M. Metchnikoff introduces a further expansion of the theory rendering it yet more complete. To this modification I shall refer in the second portion of my paper. That the theory has shown itself capable of expansion is evident by the stress now laid upon chemiotaxis as compared with the standpoint of a little more than a year ago. It will be well to state more fully the observations which have led to this expansion.

Already, in 1883, in his paper upon intracellular digestion as performed by mesoderm cells of the invertebrata, Metchnikoff noted that these did not take up indiscriminately every particle that came across their path, but exercised a choice, incepting some and leaving others untouched. And in the succeeding year, when discussing the relationship of phagocytes to the anthrax bacilli, he attributed acquired immunity to the "progressive habit exhibited by phagocytes of assimilating substances which at first they avoided."* But until some law based upon numerous determinations could be laid down as to this selective action, these observations and the explanation of them could not be regarded as adequate, and there remained a distinctly weak place in the theory.

It is to the botanists that we owe the first advance. Engelmann, in 1881, determined that certain chemical substances excite the lower motile organisms and showed that oxygen energetically attracts certain bacteria. Later, Stahl made a series of important investigations upon the plasmodium of *Æthelium septicum*, an organism living in tan pits—in infusion of oak bark. Placed on the surface of a glass moistened with water, this plasmodium remains motionless until a drop of infusion of oak bark is allowed to fall near to it; now the plasmodium moves rapidly towards the infusion. Other liquids bring about a different result—thus, a drop of one-half per cent solution of glucose, placed so as to be just in contact with the organism, causes it to move rapidly away from the drop; and so it is with solutions of different salts. What is of especial interest is that Stahl found that if this experiment with, for example, the glucose solution, be repeated frequently, the plasmodium no longer flies away; but, on

* Metchnikoff, *Virchow's Archiv*, Bd. XCVII, p. 518, 1884.

the contrary, moves, at last, towards the liquid, just as it does primarily in the case of the drop of infusion of oak bark.

Passing over certain observations of de Bary we come to numerous researches made since 1885, by W. Pfeffer, to whom especially we owe our knowledge of chemiotaxis among the lower plant forms; while equally important though not so extensive are the researches of Rosen, who pointed out that the spores of *Chytridium zygnetis* are attracted by the products of decomposition of the cells of *Zygnema*; of Zopf, upon the attraction of zoöspores of certain Chytridiaceæ towards pollen grains, and of Stange upon the chemiotactic movements of Myxomycetes and the zoöspores of *Saprolegnia*.

Pfeffer showed that certain substances exert an almost specific action upon some vegetable organisms; that, for instance, malic acid has a remarkable attraction for the seminal filaments of ferns and selaginellas; that nutritive media exert a similar action upon motile bacteria; and, again, that this chemiotaxis is determined by the specific chemical nature of the substance, and not by movements of diffusion of the liquid. Continuing his observations upon chemiotaxis in connection with bacteria, ciliate infusoria and volvocineæ, Pfeffer made out that a positive chemiotaxis is exerted by peptone (energetic), asparagin, creatin, taurin, sarcin, carnin, urea (feeble), dextrin, and for certain organisms (and here to a high degree), glucose; also in another class of substances by salicylate of sodium and morphine; while a negative chemiotaxis occurs with free acids, alkalies and salts (lactate of iron and zinc sulphate).

If then the lowest organisms exhibit such chemiotactic properties, it became a matter of considerable interest to observe whether, by using similar means, like peculiarities could be determined for the leucocytes—cells whose every property finds its counterpart among those possessed by the amoeboid animal and vegetable organisms. The first definite observations made in this direction were those of Pechelharing in 1889.* Introducing under the skins of frogs small masses of cotton wool, some moistened with indifferent fluids, others with anthrax cultures (the frog being refractory to anthrax), and removing these after some hours, Pechelharing found that the wool impregnated with the bacilli contained very many more leucocytes than did that steeped originally in indifferent fluids. A few months later, in February, 1890, Massart and Bordet† published what is really the first, as it is the most important, systematic research upon the excitability of the leucocytes. These observers employed what was essentially Pfeffer's method, and what was, it may be noted, but a modification of that introduced some years ago by Councilman to determine an allied question, namely the formation of pus by irritants, such

* Pechelharing. *La Semaine Médicale*, No. 22, 1889, p. 184.

† Massart et Bordet. *Journal de la Société Royale, des Sciences Médicales et Naturelles de Bruxelles*, 1890.

as turpentine and oil of mustard, in the absence of bacteria.* Into the abdominal lymph spaces of frogs they introduced capillary tubes, closed at one end, and filled with the substance whose action was to be examined. They employed for their observations cultures of *staphylococcus pyogenes albus*, and of the bacilli of fowl cholera, enteric fever, anthrax, etc., and at the end of twenty-four hours they found that masses of leucocytes had entered the tubes. Cultures of the *staphylococcus* were pre-eminent in attracting the leucocytes; the pure nutrient media, on the other hand, had comparatively little attractive power; hence it is the bacteria or their products which call into play a positive chemiotaxis. That it is the soluble products was shown by employing the sterilised fluids from which the microbes had been removed by filtration. Thus Massart and Bordet concluded that the products of bacterial metabolism attract leucocytes towards the focus of infection. They further discovered that chloroform and chloral hydrate temporarily suspend the movement of the wandering cells towards tubes containing positively chemiotactic media.

The work of these two observers has been extended by others, notably by Gabritchevski,† working under Metchnikoff. The effects of various substances was determined upon frogs and rabbits, fine glass tubes being placed under the skin of the first, and in the tissue of the ear of the other animals. These were extracted in twenty-four hours or less, their contents driven out on to a slide, a little weak methylene blue added, and thus the number of contained leucocytes could be approximately determined. Gabritchevski found that bacteria and their sterilised products set up an equally active positive chemiotaxis, while tubes containing other substances attracted comparatively few leucocytes. An exception to this rule was found in the case of the macerated rabbit's spleen, which, in the rabbit, exerted intense positive chemiotaxis—a fact of some small interest in connection with the bactericidal properties of the spleen, and with Hankin's observations upon the part which splenic extract can play under certain conditions in the prevention of disease.‡ Neither the mechanical irritation induced by the presence of the tubes nor the varying concentration of the contents can suffice to explain the great differences observed. The former varied but little, and with regard to the latter Gabritchevski noted that ten per cent solutions of various salts, which must necessarily have induced much more active diffusion currents than the broth serving as culture medium for the bacteria, brought about a negative chemiotaxis, with paucity of leucocytic tube contents. Chemio-

* Indeed the conflicting results gained by various observers in this connection are now explicable when we know that a given substance exerts a positive chemiotaxis in one animal—the dog, for instance—so leading to an inflammation rich in leucocytes, a negative chemiotaxis in another—as for example, the rabbit—there producing but a serous inflammation.

† Gabritchevski. "Sur les propriétés chimiotactiques des leucocytes."—*Annales de l'Institut Pasteur*, IV., 1890, p. 346.

‡ Hankin. *British Medical Journal*, July 12th, 1890.

taxis—the attraction or repulsion of the wandering cells—is alone sufficient to explain the results obtained. And this chemiotaxis varies with each species. Thus the number of leucocytes entering the tubes under the same conditions is ten times as much in the rabbit as in the frog, and whereas papayotone is indifferent in its manifestation in the latter, it is in the former positively chemiotactic. What is more, individuals of the same species show distinct variations.

All bacteria and their products are not positively chemiotactic. For example, it was found that, among those experimented with, the bacillus of fowl cholera led to a negative chemiotaxis (when fresh cultures were taken), while the observations of others would lead to the belief that Gabritchevsky employed old or attenuated cultures of the staphylococcus pyogenes, for, as he himself quotes from Steinhaus in another portion of his paper, this microbe inoculated into rabbits ordinarily causes no supuration, no collection of leucocytes, but rather a repulsion of the same, and yet Gabritchevsky determined that in this animal it produces a distinct positive chemiotaxis.*

It is unnecessary to give the data of other workers in the same field, for they substantially confirm what has been said above. Enough evidence has been brought forward to show that this attraction or repulsion of the leucocytes exercised by the pathogenic bacteria and their products must be a highly important factor in the prevention or spread of bacterial growth within the system.

But whether these chemiotactic phenomena can bear the weight thrown upon them in Metchnikoff's theory is a matter which I am inclined to think cannot be definitely decided until still more work has been accomplished upon the subject. Undoubtedly, at first sight, chemiotaxis would seem qualified to elucidate every difficulty of a certain order. It is fitted to explain how it happens that under one set of conditions ample phagocytosis manifests itself, under another, the phagocytes are conspicuous by their absence. Yet it has still to be shown that the inception of bacteria by the attracted leucocytes is in direct relation to the force of attraction. It is quite a possible case that the bacterial products may, under special conditions, attract powerfully the wandering cells, and yet that these show little inclination to incept the bacteria, the disease thus extending in spite of the positive chemiotaxis. The validity of this contention is shown by certain observations, as yet unpublished, to which Mr. Hankin very kindly

* From his observations, Gabritchevsky makes the following classification :—(1) Possessing a negative chemiotaxis : 10 per cent solution of sodium and potassium salts, lactic acid, alcohol-chloroform (watery solution), jequirity (2 per cent), glycerine, bile, bacillus of fowl cholera. (2) Indifferent, exercising a faint attraction : distilled water, weak solution of sodium and potassium salts, carbolic acid, antipyrine (1 per cent), phloridzine (1 per cent), glycogen (1 per cent), peptone (1 per cent), beef broth, blood, aqueous humour, carmine in suspension. (3) Possessing a positive chemiotaxis : sterilised and non-sterilised cultures of pathogenic and non-pathogenic bacteria—staphylococcus, B. pyocyaneus, B. anthracis, B. typhoid abd., B. prodigiosus, etc.

permits me to refer. He finds that the serum of a young rat has just as much power of attracting the leucocytes of the mouse as serum coming from an adult rat. Nevertheless, the latter has the power of preventing the development of anthrax in mice. Serum from a very young rat can exhibit no such action. While acknowledging that the evidence is now strongly in favour of Metchnikoff's contention that a negative may eventually be changed to a positive chemiotactic state, it is hard to see in this a complete elucidation of sundry difficulties. Take, for instance, a case that Metchnikoff has made his own—the case of recurrent fever. Here, during the access of the fever, spirilla swarm in the blood, while none are to be discovered within the leucocytes or other phagocytes; the crisis occurs, and in a very few hours, not a single free spirillum is discernible, but a fair number are to be seen within the cells of the spleen, there undergoing degeneration. It is difficult to comprehend that a negative has been so abruptly changed into a positive chemiotaxis. It is difficult not to opine that there is some factor added to and beyond chemiotaxis and phagocytosis, determining the crisis in acute zymotic disease. I have already laid stress on the fact that Metchnikoff freely admits the possible action of some other factor or factors not as yet included in his philosophy, and here it seems to me that other factors must be invoked. Otherwise, the more one studies the phagocyte theory and compares it with those which have been brought forward within the last year or two to replace or augment it, the more, as I shall proceed to show, one sees that it fulfils the conditions of a good theory and embraces the widest circle of phenomena.

II.

The literature which has owed its origin to Metchnikoff's studies upon phagocytosis and immunity has already attained to such proportions that it is impossible within the limits of a comparatively short article to do more than touch upon some of the main points that have been raised, and to attempt to show wherein is the strength and wherein the weakness of the counter theories.

Undoubtedly phagocytes are not always readily determinable. Leucocytes in general, as all know who have studied films of pure blood under the microscope, break up with extreme ease. Thus, unless special precautions are taken, and special methods employed, it is quite possible to see only free micro-organisms in the field, when, previous to transmission to the slide, all had been confined within phagocytes. Thus, whereas a few years ago there were many prepared to deny that phagocytosis occurred, now, when the methods to be employed are more

fully understood, there are few or none who deny its frequent occurrence, and the question is not so much whether phagocytosis plays a part in the prevention of disease, as whether it plays the most important part—whether there are not other factors equally or more important.

It is but natural that when the phagocytosis observed was insufficient to account for the number of microbes that had undergone destruction, attention should have been turned to the possible bactericidal action of the blood plasma, and of the humours of the body in which this destruction had taken place. And many workers were led to turn their enquiries into this direction, first among whom may be mentioned von Fodor, Emmerich and di Mattei, Nuttall, Buchner, Nissen, and Lubarsch. In 1887 von Fodor* found a gradual diminution in the number of bacilli to be obtained by making successive plate cultures at short intervals after inoculating sterile rabbits' blood with growths of anthrax. The blood evidently exercised a bactericidal property. In the following year Nuttall† published some much more extensive and satisfactory observations. Taking the blood serum of the frog in the first place, and afterwards employing that of a long series of animals, including man, he showed that by carefully observing the micro-organisms immersed in the fluids upon the warm stage of the microscope it was possible to follow the gradual degeneration and death of the micro-organisms in the absence of any cellular action. He found that a temperature of 55° C. destroyed the bacteria-killing power, and was of the opinion that serum and other body fluids owed their bactericidal effect to the presence of some ferment.

This paper was followed by another from the same laboratory at Breslau, in which Nissen‡ showed that numerous other pathogenic and non-pathogenic bacteria were destroyed by defibrinated rabbits' blood, as for example, the spirillum of cholera, the typhoid bacillus, Friedländer's pneumonia bacillus, and the coccus aquatilis, while others were little affected and some grew abundantly. Such were, among others, the pyogenic staphylococci and the microbes of fowl cholera and of swine erysipelas. Some of the former were killed so rapidly that Nissen concluded that the swiftness of their destruction outside the body was in itself an argument against phagocytosis, and instituted experiments to show that a similar swift destruction occurs within the blood vessels of the organism—but inasmuch as he made no series of cultures from the organs in whose capillaries the injected microbes might have been arrested, his results are, in this respect, of but little value. Emmerich

* von Fodor.—*Deutsche med. Wochenschrift*, 1887, p. 745.

† Nuttall. "Experimente über die bakterienfeindlichen Einflüsse des thierischen Körpers."—*Zeitschr. f. Hygiene*. Vol. IV., 1888, p. 353.

‡ Nissen. "Die bakterienvernichtende Eigenschaft des Blutes."—*Zeitschr. f. Hygiene*, Vol. VI., 1889, p. 487.

and di Mattei,* had, some months previously, argued that the rapidity with which the injected micrococci of swine erysipelas disappear from the blood is evidence that there can be no question of phagocytic action, and that therefore there must be some anti-bacterial substance developed by the cells of the body and free in the humours.

There is not space here to give in detail Metchnikoff's very able refutation of the argument of Emmerich and di Mattei; suffice to say that using still smaller quantities of the virus than did these observers, and employing refractory and not susceptible animals, thus again making his case stronger, Metchnikoff showed that in place of the microbes being all destroyed in from fifteen to twenty minutes some at least were still alive and capable of giving cultures after several hours, indeed for as long a period as four days, and he found that in two and a half hours there was already definite phagocytosis observable, some phagocytes containing as many as twenty-eight bacilli.†

These observations and others of a similar nature showed conclusively that outside the body the humours have an unmistakable power of destroying many forms of micro-organisms. The next question to be determined in order to establish the humoral theory was whether any relationship is to be made out between the insusceptibility of an animal to a given disease, and the degree of bactericidal power of its body fluids.

It was discovered, in the first place, that the different fluids of the body possess bactericidal powers differing in degree. Thus, for example, the blood serum is generally speaking the most active of all, the aqueous humour the least active. But the fact is not opposed to the humoral theory, for in the susceptible animal it requires a larger injection of pathogenic microbes into the circulation to induce disease than is required in the case of inoculation into the anterior chamber of the eye. At the same time, the fact that the aqueous humour is deficient in leucocytes, while the blood contains them in quantity, makes this argument support the phagocyte theory equally well. It is when we attempt to solve the problem more directly that the humoral theory is found to be insufficient, and we owe to those who first propounded the theory the most striking demonstrations of its insufficiency. Thus Nuttall showed that the blood serum of the susceptible rabbit is more bactericidal towards the anthrax bacilli than is that of the sheep which has been rendered immune, and Behring and Nissen‡ pointed out that the same micro-organism develops without delay in the serum of such refractory animals as fowls, frogs, cats, and sheep that have been rendered immune,

* Emmerich and di Mattei.—*Fortschritte der Medicin*, VI. p. 729.

† "In the torpedo, injecting anthrax bacilli, Lubarsch found phagocytes after three quarters of an hour in pretty nearly every preparation he made, many phagocytes containing from 20 to 30 bacilli."—*Ctbl. f. Bakt.*, Vol. VI., 1889, p. 536.

‡ Behring and Nissen.—*Zeitschr. f. Hygiene*, Vol. VIII., 1890, p. 424.

is variable in its development in the susceptible rabbit and the refractory dog, and is only surely stopped in its development and destroyed by the serum of the rat—a very insusceptible animal. To these observations may be added those of Charrin and Roger,* who found that the rabbit is highly refractory to quarter evil, the guinea-pig most susceptible, yet the microbe of the disease grows more easily in the rabbit's than in the guinea-pig's blood serum. In his studies upon the action of the rat's serum Metchnikoff† added a still more adverse case. He discovered that the blood serum of a rat which had succumbed to anthrax had bactericidal powers equal to that of a rat immune to the disease. Evidently, therefore, there is no constant relationship to be made out between the extent to which an animal is refractory and the degree of the bactericidal power possessed by its blood-serum. Indeed, few satisfactory cases have been brought forward in support of such relationship. It has been found that the vibrio Metchnikovi is not killed by the serum of susceptible guinea-pigs, is killed by that of guinea-pigs which have been made refractory‡; again, that while the growth of the quarter evil microbe is, as above stated, not abundant in guinea-pigs' serum, it is still more scanty in that of the vaccinated guinea-pig§ and that in the serum of rabbits made refractory towards erysipelas there is as abundant a growth of the streptococcus as in that of normal rabbits, but now the coccus becomes attenuated,¶ and similar phenomena are observed with the *B. pyocyaneus* in rabbits' serum. One or two more examples might be given, but still not enough to prevent there being more exceptions than illustrations in case we attempt to propound a law as to the direct relationship between immunity and the bactericidal power of the body fluids.

A further difficulty, to which attention has already been called, is the fact that the destruction of microbes in the extracted body fluids is much more rapid than in the humours within the organism. Not only is it more rapid, but it is more extensive. Thus Lubarsch|| observed that in order to kill a rabbit at least 16,000 virulent anthrax bacilli are required if the injection be made intravenously—directly into the circulating blood; a lesser quantity, 10,000, for example, only causes a transient disturbance; or otherwise, the whole circulating blood can only destroy 10,000 bacilli or so at a time. Now, one cubic centimetre of rabbits' blood serum can destroy in a few minutes an equal or even much greater amount.

* Charrin and Roger.—*Comptes rendus de la Société de Biologie*, 1889.

† Metchnikoff.—*Annales de l'Institut Pasteur*, 1890, p. 193.

‡ Behring and Nissen.—*Loc. cit.* Confirmed by Metchnikoff, *Annales de l'Institut Pasteur*, 1891, September number.

§ Charrin and Roger.—*Comptes rendus de la Société de Biologie*, 1890.

¶ Roger. See Bouchard. "Essai d'une Théorie de l'Infection."—X. Internat. Med. Congress, 1890.

|| Lubarsch.—*Centralblatt. f. Bacteriologie.*, VI., 1889, p. 841.

Here, then, there is a remarkable want of relationship between the action of the humours outside the body and within. We are forced to acknowledge that if a factor at all in the production of immunity, the bactericidal property of the humours is not of primary importance, and the next question to be dealt with is how to reconcile the undoubted action "in vitro" with what occurs "in corpore."

A most important accompaniment of the clotting of blood and the production of serum is the breaking down of leucocytes, and, as in all the lymph spaces of the body, leucocytes can, and do, enter, so all the body fluids (as distinguished from the secretions) contain a larger or smaller number of these cells—the blood and lymph the most, the aqueous humour the fewest. Thus all of these body fluids when removed become modified in that now the leucocytes break down and their soluble contents become set free. Can this account for the bactericidal properties of the serum and other fluids?

Curiously enough, those who first called attention to these properties were the first to adduce facts in favour of this supposition. Buchner* who, from the beginning, acknowledged that his demonstration of the bacteria-killing power of blood serum did not refute, but only modified, the phagocyte theory, showed that if the cells and proteid matter of defibrinated blood were, by keeping, allowed to fall, the serum lost its power, while the layer of corpuscles at the bottom of the vessel possessed it. And Nissen† unwittingly brought forward what is as strong a proof as could be desired in favour of the same contention, a proof to which I have seen no reference, and which I would, therefore, dwell upon in some detail. Blood may be prevented from clotting either by the action of peptone or by adding to it a solution of magnesium sulphate. In the former case, peptone plasma, as Nissen shows, destroys leucocytes, and this peptone plasma has bactericidal properties as strong, or almost as strong, as ordinary serum; in the latter case the leucocytes remain intact, but the plasma has absolutely no destructive influence. And so Nissen argued, that in one case the destruction is undergone in the absence of leucocytes, in the other, although leucocytes are present in abundance, no bacteria are killed, hence there is here a proof positive of the correctness of the humoral theory as opposed to that of phagocytosis. But what the experiment proves, and proves most prettily, is that where the leucocytes are prevented from breaking down and liberating their contents, there the plasma has little or no bactericidal action, and that thus the bacteria-killing property of plasma or serum is due to the dissolution of leucocytes.

* Buchner.—*Centralblatt f. Bakteriologie*, 1888, Vol. V. p. 817, and Vol. VI. p. 1 and p. 561, and *Archiv für Hygiene*, Vol. X., 1890, p. 130.

† Nissen.—*Loc. cit.*, p. 500.

Many observers have shown that the intracellular digestion of the lower organisms is brought about by the formation of vacuoles around the food particles, these vacuoles being filled with a digestive fluid whereby the food is broken up and rendered soluble and assimilable. In the phagocytes, as Metchnikoff pointed out years ago, a similar process obtains in relation to the incepted bacteria when these are destroyed. So that clearly the phagocytes, under favourable conditions, elaborate substances capable of killing and breaking up the microbes.

This view that the leucocytes contain a bactericidal substance or substances is strengthened, if it be not fully established, by Hankin's* discovery of a bacteria-killing globulin present in the lymphatic glands of the dog and cat, and also obtainable from the spleen. The former organs and, to a large extent, the latter are composed of what may be termed potential phagocytes.

We thus arrive at the conclusion that the leucocytes and probably other mesodermal cells function as phagocytes under appropriate conditions within the organisms, and kill, or it may be, only modify the microbe by means of their digestive fluids; that outside the organism, where there has been destruction of the leucocytes, the soluble constituents of these cells are liberated into the blood serum, lymph, etc., which now gain the power of killing or modifying the microbes present.

It is to the fact that Metchnikoff is now prepared to receive this doctrine and to extend his theory in this direction that I referred in the earlier part of this paper, for, in his last communication,† he shows that the same attenuation and alteration in the mode of growth of the vibrio Metchnikovi is observable when the vibrio has been acted upon by phagocytes within the body of "vaccinated" guinea-pigs, and when it has been acted upon outside the body by the blood serum of like animals. He points out that in the latter case the change may be due to substances derived from the broken down leucocytes.

Having arrived thus far, the next problem to be dealt with is whether the dissolution of leucocytes within the organism is capable of aiding the action of the intact leucocytes and other phagocytes in preventing the growth and extension of pathogenic microbes. Such dissolution is, we know, of constant occurrence under normal conditions. During the process of digestion, for example, not only is there a very definite increase in the number of the white corpuscles of the blood, but as Heidenhain has shown, there is a most active breaking down of the same, and in the course of infectious disease it would seem that the production and destruction is largely increased wherever there is a well-marked reaction on the part of the system. That there is increased formation is evidenced by the proverbial "buffy coat" of febrile blood in certain states; and having

* Hankin.—*Proceedings of the Royal Society*, Vol. XLVIII, 1890, p. 93.

† Metchnikoff.—*Annales de l'Institut Pasteur*, 1891, No. 8.

granted the interaction between the micro-organism and the leucocyte, we may legitimately admit that there is destruction.* In addition, therefore, to exciting the chemiotactic properties of the white corpuscles, the "toxines" of pathogenic bacteria may produce a proliferation of the leucocytes in the lymphatic glands. Apart from the old world proof of this proliferation, there is the more recent proof supplied by Buchner† and Tchistovitch.‡ Thus here is provided an additional argument in favour of regarding the leucocytes as important factors in the prevention of disease.

But when we come to examine more nearly into the facts for and against the possession of bactericidal powers by the body fluids within the organisms, we find that there is so far a want of conclusive evidence in favour of assuming that it exists. The statements of Emmerich and di Mattei, of Nissen, and others, that bacteria injected into the circulation are destroyed more rapidly than could be accounted for by phagocytosis, have, as I have shown, been shaken by Metchnikoff. Lubarsch, a very careful observer, states certainly that the spores of anthrax are inhibited from developing in the frog, whether they be enclosed within cells or free in the lymph; and more recently Sanarelli§ has, by placing anthrax bacilli in small closed tubes formed of collodion, and filled with lymph which had diffused through the walls when the tubes were placed in the subcutaneous lymph sac of frogs, shown that these undergo alteration in consequence of the action upon them of the lymph, free from cellular elements. If this be correct, it only shows that the body fluids have a destructive action much feebler than that possessed by the leucocytes.

In this connection may be mentioned Bouchard's observations upon the bacillus pyocyaneus and his theory advanced at the Berlin Congress that immunity is primarily due to the condition of the humours. Pathogenic bacteria, according to him, give rise to substances which hinder the inflammatory process, and it is only when these substances are inadequately represented, or when yet other bacterial products have stimulated the cells, that the cells intervene. The organism rendered refractory becomes an unfavourable soil for the production of these inhibitory bodies, and in consequence of this altered condition of the humours inflammation occurs, free emigration of the leucocytes takes place, and phagocytosis ensues. But this theory unfortunately does not embrace all cases. Many very virulent diseases cause well-marked inflammatory changes, and it may be laid down that in a large number of acute diseases, what we have to deal with is not an absence but a modi-

* See also Capparelli.—*Centralblatt f. Bakteriologie*, Vol. X., 1891, p. 277.

† Buchner.—*Münchener med. Wochenschr.*, 1890, No. 47.

‡ Tchistovitch.—*Annales de l'Institut Pasteur*. July, 1891.

§ Sanarelli.—*Centralblatt f. Bakteriologie*, Vol. IX., 1891, Nos. 14-16.

fication of the inflammatory process. Thus, if we inoculate a rabbit in the one ear with attenuated, in the other with virulent, anthrax, we find that in the course of a few hours the latter ear exhibits much more evident signs of inflammation than does the former; it is much more swollen, and there is greater congestion, and now if we examine the two ears we find that in the latter we have to deal with a serous inflammation; there has been great serous exudation. In the former there is less marked serous exudation, but an extensive emigration of leucocytes; the inflammatory process is distinctly of a cellular nature.* Therefore, it can safely be said that the condition of the humours as permitting or preventing the inflammatory reaction is not, in a large number of cases, capable of explaining the phenomena attendant upon the production of immunity and the spread of disease.

Returning now to a more direct discussion of the problem before us, we may say that the weight of evidence is against the assumption that the body fluids in the refractory animal possess any but very feeble bactericidal powers. The numerous instances that have been brought forward of pathogenic micro-organisms retaining their vitality, and even their virulence for hours and days after they have been injected under the skin or into the anterior chamber of the eye of definitely refractory animals prevent any other conclusion.

Yet it seems to me that there is a class of cases in which there may be development or exaltation of these bacteria-killing powers. The instances referred to above are all of them cases in which purely local inoculations have been made, or where very acute generalised infective process has been set up. It is the generalised infective processes of slower progress that clinically possess the greatest interest. Let us study pneumonia, for example. Tchistovitch found that in those cases in which, in consequence of the strength of the virus, a fatal ending was inevitable, there no increase occurred in the white corpuscles present in the blood;† where a less powerful virus was inoculated, there the increase was most marked. Unless it so happens that within the organism the dissolution of the white corpuscles is followed by immediate destruction or inaction by other cells of their bactericidal substances, it seems eminently probable that here the blood plasma and the lymph must gain bactericidal powers; and I would go so far as to suggest that the assumption that these bactericidal powers become increased helps to explain the resolution of such disease by crisis. If the strength of the virus and the reactive powers on the part of the organism are such that the bacterial products induce a proliferation of leucocytes

* Metchnikoff. *British Medical Journal*, Jan. 31st, 1891.

† With this may be compared Hildebrandt's observation that the febrile state induced by the injection of lethal doses of various ferments is accompanied by a diminution in the number of white corpuscles in the blood, a diminution which would seem to be due to active destruction of the corpuscles.—*Virchow's Archiv*, Vol. CXXI., 1890, p. 1.

rather than an inhibition of their development, then, along with the increased production an increased destruction may be predicated. I have already pointed out that the products of splenic cells—of phagocytes and potential phagocytes—cause positive chemiotaxis, that is to say, stimulate intact phagocytes. At the same time they are, in the free condition, capable of interfering with the growth of pathogenic micro-organisms. If, then, through the breaking down of leucocytes in the blood and lymph, there be a liberation of these substances and gradual accumulation of the same, it is not advancing too bold a theory to suggest that there will be a moment at which the bacteria are so depressed in their vital powers, and the leucocytes and other mesodermal cells so stimulated, that now they are capable of functioning actively as phagocytes, and so of bringing the morbid process to a critical termination.

Without doubt it is difficult to pass satisfactorily through the labyrinth of facts bearing upon the subject of immunity, there are so many side issues, so many paths that promise well at first and which when pursued end in a *cul de sac*. While, therefore, it is impossible to say as yet that this labyrinth can be completely mastered, I shall be satisfied if I have shown that the way has been further advanced during the last few years towards arrival at the central truth. From what I have stated it would seem evident that phagocytosis is a most important, and very possibly the most important, factor in the prevention of infective disease, and that it is aided by other factors, factors largely dependent upon the activity of the white corpuscles and certain other mesodermal cells. That these cells and their products act also upon the products of bacterial growth is also evident from certain recently-published observations. But this and the researches of the last few months upon the modes of producing what may be termed chemical vaccination is a subject so large that it is not possible to deal with it here; its consideration must be left to a later date when possibly new facts will place the matter in a less uncertain light.

